

**We claim:**

- 1) A *Mycobacterium* strain with a modified tyrosine phosphatase gene in its genome, wherein the said *Mycobacterium* strain is incapable of expressing the active tyrosine phosphatase gene.
- 5 2) The *Mycobacterium* strain as claimed in claim 1 wherein the *Mycobacterium* species is selected from a group consisting of *M. tuberculosis* and *M. bovis*.
- 3) The *Mycobacterium* strain as claimed in claim 1 wherein the modified tyrosine phosphatase gene is modified *mptpA* gene.
- 4) The *Mycobacterium* strain as claimed in claim 3 wherein the modified *mptpA* gene is as shown in SEQ ID NO : 15.
- 10 5) The *Mycobacterium* strain as claimed in claim 1 wherein the modified tyrosine phosphatase gene is modified *mptpB* gene.
- 6) The *Mycobacterium* strain as claimed in claim 5 wherein the modified *mptpB* gene is as shown in SEQ ID NO : 16.
- 15 7) A recombinant vector comprising the modified *mptpA* gene of claim 3.
- 8) A recombinant vector as claimed in claim 7 is pAKΔA.
- 9) A recombinant vector comprising the modified *mptpB* gene of claim 5.
- 10) A recombinant vector as claimed in claim 9 is pBKΔB.
- 20 11) The recombinant vector as claimed in claim 7, wherein the nucleotide sequence of *mptpA* gene as shown in SEQ ID NO: 11 is modified.
- 12) The recombinant vector as claimed in claim 9, wherein the nucleotide sequence of *mptpB* gene as shown in SEQ ID NO: 12 is modified.
- 13) The recombinant vector as claimed in claim 7 or 9, wherein the *mptpA* or *mptpB* gene is modified by insertion, deletion, mutation or substitution.
- 25 14) The recombinant vector as claimed in claim 7 or 9, wherein the *mptpA* or *mptpB* gene is modified by substituting an internal region of the *mptpA* or *mptpB* gene by an antibiotic resistance marker gene.
- 15) The recombinant vector as claimed in claim 14, wherein the antibiotic resistance marker gene imparts resistance to either hygromycin or chloramphenicol preferably to hygromycin.
- 30 16) The recombinant vector as claimed in claim 7 or 9, wherein a second antibiotic marker gene is inserted in the backbone of the said recombinant vector.
- 17) The recombinant vector as claimed in claim 16, wherein the second antibiotic marker gene imparts resistance to kanamycin or gentamycin.
- 35 18) An isolated nucleotide sequence of the *mptpA* gene encoding the mycobacterial tyrosine phosphatase A as shown in SEQ ID NO : 11.

- 19) An isolated nucleotide sequence of the *mptpB* gene encoding the mycobacterial tyrosine phosphatase B as shown in SEQ ID NO : 12.
- 20) An isolated nucleotide sequence of the modified *mptpA* gene as shown in SEQ ID NO : 15.
- 5 21) An isolated nucleotide sequence of the modified *mptpB* gene as shown in SEQ ID NO : 16.
- 22) A method for developing a *Mycobacterium* strain with a modified tyrosine phosphatase gene in its genome comprising the following steps:
  - a. extracting genomic DNA from *Mycobacterium* strain,
  - 10 b. amplifying the tyrosine phosphatase gene along with the flanking sequences using specific primers from the genomic DNA of step (a) to obtain a DNA fragment,
  - c. characterizing the fragment of step (b),
  - d. cloning the fragment of step (b) in a non-replicative vector,
  - 15 e. modifying the fragment in the non-replicative vector of step (d),
  - f. inserting an antibiotic resistance marker gene within the fragment of step (e) to obtain a non-replicative vector containing a modified tyrosine phosphatase gene,
  - g. cloning of a second antibiotic resistance marker gene in the backbone of the non-replicative vector of step (f), to obtain a recombinant vector,
  - 20 h. introducing the recombinant vector of step (g) into *Mycobacterium strains*,
  - i. selecting for primary recombinant *Mycobacterium strains* using the first antibiotic selection marker gene,
  - 25 j. culturing the primary recombinant *Mycobacterium strains* of step (i) harboring the first antibiotic resistance marker gene,
  - k. selecting the secondary recombinant *Mycobacterium strains* of step (j) that is sensitive to the second antibiotic resistance gene present in the vector backbone,
  - 30 l. culturing the secondary recombinant *Mycobacterium strains* of step (k), wherein the said recombinant *Mycobacterium* strain harboring the modified tyrosine phosphatase gene which shows defective growth in activated macrophages and animals.
- 23) The method as claimed in claim 22, wherein the *Mycobacterium* species is selected from a group consisting of *M. tuberculosis* and *M. bovis*.

24) The method as claimed in claim 22, wherein in step (b) the specific primers are selected from a group comprising of SEQ ID NO : 1 to 4 for amplification of *mptpA* along with its flanking regions and SEQ ID NO : 5 to 8 for amplification of *mptpB* along with its flanking regions.

5 25) The method as claimed in claim 22, wherein in step (b) the tyrosine phosphatase gene is *mptpA* gene as shown in SEQ ID NO : 11.

26) The method as claimed in claim 22, wherein in step (b) the tyrosine phosphatase gene is *mptpB* gene as shown in SEQ ID NO : 12.

27) The method as claimed in claim 22, wherein in step (b) the DNA fragment is a sequence as shown in SEQ ID NO : 13.

10 28) The method as claimed in claim 22, wherein in step (b) the DNA fragment is a sequence as shown in SEQ ID NO : 14.

29) The method as claimed in claim 22, wherein in step (c) the DNA fragment is characterized by sequencing and restriction enzyme analysis.

15 30) The method as claimed in claim 22, wherein in step (f) the modified tyrosine phosphatase gene is modified *mptpA* gene as shown in SEQ ID NO : 15.

31) The method as claimed in claim 22, wherein in step (f) the modified tyrosine phosphatase gene is modified *mptpB* gene as shown in SEQ ID NO : 16.

32) The method as claimed in claim 30 or 31, wherein the *mptpA* or *mptpB* gene is modified by insertion, deletion, mutation or substitution.

20 33) The method as claimed in claim 30 or 31, wherein the *mptpA* or *mptpB* gene is modified by substituting an internal region of the *mptpA* or *mptpB* gene by an antibiotic resistance marker gene.

34) The method as claimed in claim 33, wherein the antibiotic resistance marker gene imparts resistance to either hygromycin or chloramphenicol preferably to hygromycin.

25 35) The method as claimed in claim 22, wherein in step (g) the second antibiotic marker gene imparts resistance to kanamycin.

36) The method as claimed in claim 22, wherein in step (g) the recombinant vector is either pAKΔA or pBKΔB.

30 37) The method as claimed in claim 22, wherein in step (h) the introduction of the vector is by either electroporation or phages.

38) The method as claimed in claim 22, wherein in step (i) the selection of primary recombinant *Mycobacterium* strain is by using either hygromycin or chloramphenicol.

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- 39) The method as claimed in claim 22, wherein in step (k) the selection of secondary recombinant *Mycobacterium* strain which are resistant to either hygromycin or chloramphenicol but sensitive to second antibiotic resistance marker (kanamycin).